tial microbiological sensor and regulator of macrophages' functions against *Mycobacterium tuberculosis.*

REPRODUCCIÓN / REPRODUCTION 1

123. (26) **COCAINE ALTERS MOUSE GERM CELLS EPIGENOME WITH DIRECT IMPACT ON H4AC EXPRESSION AND DNA METHYLATION IN THE SPERM**

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Cocaine intake is associated with testicular toxicity and significant reproductive function impairment. There is accumulating evidence that cocaine administration can trigger nongenetic inheritance through the male germ line affecting development and behavior of the offspring. The influence of environmental factors on the epigenome of male germ cells appears to be most impactful if it happens during a developmental phase when these cells are epigenetically reprogrammed. In the present study, we measured epigenetic marks in isolated germ cells of adult mice treated with cocaine (10 mg/kg) or vehicle, in an intermittent binge protocol (3 i.p. injections, 1 h apart, one day on/off for 13 days). We found that chronic cocaine intake disrupts male germ cell epigenetic homeostasis, increasing global methylated citocine (5-mC) levels in DNA from germ cells and cauda epididymal sperm (germ cells: vehicle 13.15 ± 0.99 vs cocaine 20.113 ± 2.28; sperm: vehicle 15.81 ± 1.08 vs cocaine 21.35 ± 1.52). Cocaine also increased acetylated histone 4 (H4ac) protein levels and decreased class I deacetylases HDAC1/2 mRNA and protein expression ($p<0.05$). The mRNA expression levels of class IIa and IIb HDACs were also altered ($p<0.05$). Immunolocalization studies showed that HDAC1/2 were mainly expressed in primary spermatocytes and H4ac was immunolocalized in late meiotic stages in vehicle mice while it was detected in primary spermatocytes and in successive stages until round spermatid in cocaine-treated mice. We observed altered mRNA expression of DNA methylation markers in isolated germ cells showing decreased levels of Dnmt3b and Tet1 gene expression after cocaine treatment ($p<0.05$). TET1 was mainly immunolocalized in primary spermatocytes in vehicle and cocaine-treated mice. The results presented here broaden the basic knowledge of the impact of addictive stimulants on testicular pathophysiology, fertility and male reproductive health and imply that altered epigenetic homeostasis by cocaine may have potential consequences on future generations.

124. (592) **ANANDAMIDE IMPAIRS THE SYNCYTIALIZATION OF HUMAN CYTOTROPHOBLAST CELLS**

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The syncytiotrophoblast (STB) is the main structural and functional layer of the human placenta. The underlying cytotrophoblast cells (CTB) fuse to form this multinucleated syncytium in a process called syncytialization. Disturbance in this event is associated with pregnancy pathologies such as preeclampsia and intrauterine growth restriction.
Endocannabinoids (eCBs) are a group of bioactive lipid mediators which, together with their receptors and the enzymes involved in their metabolism, constitute the endocannabinoid system (ECS). Anandamide (N-arachidonylethanoalmine, AEA) and 2-Arachidonoylglycerol (2-AG) are the major eCBs, having both important roles in human placentation. Recently it has been reported that 2-AG impairs CTB fusion. However, the role of AEA in this process remains unknown.
The aim of this work was to study the impact of AEA on syncytialization and to elucidate the mechanisms involved in this process.
BeWo cell line was cultured with 25μM forskolin (FSK) to induce syncytialization. CTB (BeWo) and STB (BeWo+FSK) were treated with or without R-(+)-Methanandamide (0.01μM-10μM Met-AEA), a stable analogous of AEA. Cell viability was evaluated by MTT assay and LDH activity. We studied different trophoblast syncytialization markers: Glial cells missing-1 (GCM-1) mRNA levels by RT-qPCR; human chorionic gonadotropin (hCG) concentration by chemiluminescence immunoassay; syncytin-1 protein expression, cell size and DNA content by flow cytometry; and E-cadherin distribution by immunocytochemistry. Incubation with 1μM Met-AEA diminished the mRNA levels of GCM-1($p<0.05$ n=4) and the secretion of hCG ($p<0.05$ n=3), syncytialization markers increased by the forskolin-induced cell fusion. This was partially reverted by incubation with AM630, an antagonist of cannabinoid receptor 2. Additionally, Met-AEA produced a decrease in cell fusion evidenced by E-cadherin distribution and impaired the increase in the number of syncytin-1-positive cells stimulated by FSK.
These results suggest that increased AEA levels may disturb human trophoblast syncytialization.

125. (704) **THE ENDOCANNABINOID SYSTEM IS PRESENT IN THE CERVIX OF PREGNANT MICE AND IS INVOLVED IN PRETERM LABOR INDUCED BY LIPOPOLYSACCHARIDE ADMINISTRATION.**

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The endocannabinoid system (ECS) is one of the numerous signaling pathways that have been involved in the pathophysiology of pregnancy. In our laboratory, we have previously demonstrated the presence of the ECS in implantation sites and infiltrating immune cells in a murine model of lipopolysaccharide (LPS)-induced embryonic resorption. On the other hand, in an in-vivo LPS-induced preterm labor model, we have observed that the immune challenge produced an augmentation of matrix metalloproteinase (MMP) activity in the cervix. Based on these premises, the objective of the present study was to investigate the presence and possible modulation of the ECS in cervical tissue in LPS-induced preterm labor model. Furthermore, we proposed to study the metalloproteinase activity changes induced by LPS and its possible modulation for the ECS in vitro. In first place we evaluated protein levels of CB1, CB2, NAPE-PLD and FAAH in cervical tissue from pregnant balb/c mice on 15 day of pregnancy, treated or not with LPS. We demonstrated the presence of all the components of de ECS in the cervix and we found that the immune challenge downregulates the expression of FAAH protein. On the other hand, we utilized an in vitro model to evaluate MMP9 and MMP2 activity. Briefly, cervix from 15 day pregnant mice were obtained and cultured in presence or not of LPS for 6 hours. MMPs gelatinase activity was evaluated by zymography. We found that LPS increased significantly MMP9 and MMP2 gelatinase activity in cervix ($P<0.05$) and preliminary data showed that an specific antagonist of CB1 could be modulating MMPs activity, reducing it to control levels. Collectively, we showed that the ECS is present in the cervical tissue and probably it is involved in cervical remodeling.

126. (677) **ENDOCANNABINOID SYSTEM CARACTERIZATION IN DECIDUAL TISSUES FROM 15-DAY PREGNANT MICE**

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Endocannabinoids, like anandamide (AEA), are the endogenous ligands for cannabinoids receptors CB1 and CB2 that are part of the endocannabinoid system (ECS). Similarly to prostaglandins, endocannabinoids are implicated in different aspects of reproduction, such as maintenance of pregnancy and parturition. In our laboratory, we have previously seen that lipopolysaccharide (LPS) systemic administration increased the level of prostaglandin F2α production and the protein level of cyclooxygenase (COX)-2 in deciduas from